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Short Communication

Pretreatment of *Miscanthus* stalk with organic alkali guanidine and amino-guanidine

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HIGHLIGHTS

- Guanidine and amino-guanidine are used to pretreat *Miscanthus* stalks.
- These catalysts are a class of environmentally friendly pretreatment catalysts.
- The IR spectra of H-bond appeared blue shift after pretreated *Miscanthus* stalks.
- *Miscanthus* is proved an excellent energy raw material.

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ABSTRACT

Organic alkali guanidine and amino-guanidine were used as catalysts to pretreat *Miscanthus* stalks. The effects of catalyst loadings, pretreatment temperature and time, on pretreatment results were studied. Between guanidines and amino-guanidines, guanidines were of benefit to produce hexose and amino-guanidines were in favor of producing pentose in stalk enzymolysis process. SEM images showed that the stalk surface after pretreatment were porous, cracked, and corroded. XRD data showed that the relative crystallinity index of cellulose after pretreatment was increased. FTIR spectra illustrated that both guanidine and amino-guanidine were effective to remove lignin and degrade hydrogen bonds of cellulose. TG data indicated that the initial temperature of rapid weight loss of *Miscanthus* stalks pretreated by the guanidine was higher than that by the amino-guanidine. The maximum sugar yields of *Miscanthus* stalks pretreated by the guanidine and the amino-guanidine after enzymolysis for 24 h were 350 and 370 mg/g stalks, respectively.

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1. Introduction

There have been enormous interests and strategic significance in recent decades in renewable energy development with raw materials of biomass to face the serious situation of growing consumption of fossil fuel and its side effects, such as greenhouse effect, acid rain, and photochemical smog (Gupta and Verma, 2015). The first generation of bio-fuel is fuel ethanol, which is produced by raw materials of starch, such as corn and wheat. But it is not possible to vigorously develop the first generation of bio-fuel, because of large scale consumption of human food. The second generation of bio-fuel, which is produced by raw materials of lignocellulose, such as crop stalks, agricultural and forestry residues, and shrubs, has recently been studied and developed all over the world (Asgher et al., 2014). The application of raw materials of non-food crops and residues is the remarkable feature of the second generation of bio-fuel (Chovau et al., 2013).

Miscanthus, as a new energy crop, has attracted considerable attentions and been an extensively studied topic (Zhang et al., 2013). *Miscanthus*, belonging to the *Miscanthus anderss* species, is a perennial rhizomatous grass with C4 photosynthetic pathway. *Miscanthus* genus includes 4 groups with 17 species. Among 6 species are found in China and distributed in the range at the south of Yangtze River (Li et al., 2013). *Miscanthus* genus is well known as one of most potential energy crops because of the characteristics of high adaptability, high bio-production, high quality of fiber, and low ash content (Han et al., 2011).

In this work, the catalytical properties of guanidines (G) [$NH = C(NH_2)_2$, $\mathrm{p}K_b^{\theta} = 1.5$] and amino-guanidines (AG) [$NH = C(NH_2)(NH)(NH_2)$, $\mathrm{p}K_b^{\theta} = 3$] to pretreat *Miscanthus* stalks were studied, and the effects of parameters on the pretreatment results and contents of pentose and hexose after fermentation were investigated as well. Several analysis techniques were employed to characterize the structure and property changes of *Miscanthus* stalks before and

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after pretreatment. A mechanism of pretreatment activities of organic guanidines on *Miscanthus* stalks was proposed. Guanidines and amino-guanidines are considered as sorts of green catalysts because their main degradation compounds are ammonia (NH_3) or ammonium ion (NH_4^+) in nature (Jahani et al., 2011), without any pollution to environment. This work would provide new insights for the preparation of various novel highly active and environmentally friendly pretreatment catalysts of biomass stalks in the future.

2. Methods

2.1. Materials and reagents

Miscanthus stalks were provided by the Biomass and Bioenergy Research Centre in Huazhong Agricultural University. The freshly reaped *Miscanthus* plant was treated by removing blades and sheaths. The remained stalks were cut to an appropriate length and enzyme deactivated in an electric air blowing dryer at 120 °C for 10 min. The deactivated stalks were dried in an oven at 60 °C till no weight loss. The dried samples were then ground, screened to powders with 40 mesh, and stored in a desiccator.

The analytically pure regents of guanidine, amino-guanidine, anthranone, and orcinol were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Instruments

The instruments include a Nicolet AVATAR330 Fourier Transform Infrared spectrometer (FTIR), USA; a Rigaku D/Max-IIIA X-ray Diffractometer (XRD), Japan; a JSM-5610LV Scanning Electron Microscope (SEM), Japan; and a Netzsch TG209 Thermogravimetric (TG) Analyzer, German.

2.3. Experimental methods

2.3.1. Pretreatment of Miscanthus stalks by alkali guanidine and enzymolysis

The stalk powder of 0.5 g was put into 15 mL plastic centrifuge tubes. 1–5% (mass fraction) alkali guanidine solution of 10 mL was then added into the centrifuge tubes and thermally treated in a thermostatic water bath at 40–80 °C for 4–8 h. The pretreated solutions in the centrifuge tubes were then centrifuged at 3000 r/min for 5 min to separate the upper liquor and the residue. The pH of the upper liquor was adjusted by sulfuric acid to 1 and lignin was precipitated. The upper liquor was diluted to measure the contents of pentose and hexose. The residue was washed by water till pH 7. HAc-NaAc buffer solution of pH 4.8 and cellulose complex enzyme with 5 wt% of stalk mass were added to the residue. The mixture was put into a constant temperature oscillator at 50 °C to be enzymolyzed for 24 h. After enzymolysis, the contents of hexose and pentose in the solution were measured. The performance was repeated three times for one sample and the average was taken as the final value.

The anthrone method (Jermyn, 1975) was used to measure the total sugar and the orcinol method (McKay, 1964) was used to measure the content of pentose.

2.3.2. Sample characterization before and after pretreatment

The change of structure and morphology, crystallinity of lignocellulose, the structure of functional groups, and the thermal properties of lingo cellulose of the stalks before and after pretreatment was characterized by SEM, XRD, FTIR, and TG tools, respectively.

3. Results and discussion

3.1. Effects of pretreatment on sugar yield in enzymolysis process

The effects of various parameters, such as catalyst loadings, pretreatment temperature, and pretreatment time, on pretreatment results were studied. The influence of catalyst loadings on sugar yield was shown in Fig. 1A. The pretreatment was performed at 60 °C for 6 h. Both catalysts loading weight were changed by 1%, 2%, 3%, 4%, and 5% (wt%, refer to the weight of *Miscanthus* stalks). The influence of different pretreatment temperatures on sugar yield was shown in Fig. 1B. The pretreatment was performed at different temperature of 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C, for 6 h. Both catalysts loading weight was 3% (wt%). The influence of different pretreatment time on sugar yield was shown in Fig. 1C. The pretreatment was performed at 60 °C for different period of time of 4 h, 5 h, 6 h, 7 h, and 8 h. Both catalysts loading weight was 3% (wt%). Results shown that the maximum sugar yields of *Miscanthus* stalks pretreated by the guanidine and the amino-guanidine after enzymolysis for 24 h were 350 and 370 mg/g stalks, respectively.

In short, the most important factor to influence the sugar yield in enzymolysis process was the loadings of pretreatment catalysts. The second important factor was the pretreatment temperature. The influence of the pretreatment time on the sugar yield was relatively slight. The guanidine catalyst relatively strongly influenced the yield of hexose and the amino-guanidine catalyst was relatively favor of the yield of pentose. The guanidine catalyst is generally better than the amino-guanidine catalyst in the pretreatment process because of its relatively strong basicity.

3.2. The measurement of crystallinity index of cellulose

The relative crystallinity index of all *Miscanthus* stalk samples after pretreatment based on Section 3.1 was measured by an X-ray diffractometer with Cu Kα radiation ($\lambda = 0.15406$), as summarized in Supplemental Table S1. The XRD analysis was operated at 35 kV and 30 mA in the 2θ range of 5–60° with a scanning step of 0.02°. A maximum intensity of diffraction peak (002) was found at $2\theta = 22.5°$ and a minimum peak was at $2\theta = 18°$. Thus, the relative crystallinity index of cellulose could be calculated by the following Segal empirical equation (Segal et al., 1959):

$$C_r\ I\% = [(I_{002} - I_{\mathrm{am}})/I_{002}] \times 100 \tag{1}$$

where $C_r\ I\%$ is the percentage of the relative crystallinity; I_{002} is the intensity of lattice plane (002), in arbitrary unit; and I_{am} is the intensity at $2\theta = 18°$, in the same unit with I_{002}.

It can be seen that, in comparison to the *Miscanthus* stalks without pretreatment, the crystallinity of *Miscanthus* stalks after pretreatment at different conditions was to some extents increased. It indicated that the boundaries between crystalline region and amorphous region of cellulose became apparent. Because of the high-speed movement of water molecules and hydroxide ions, the crystalline region, where there has crystalline defect in the molecular chains of cellulose, would quickly become smaller crystalline regions under the impact, leading to shrinkage of crystalline regions (Bansal et al., 2010). Lignin encapsulation surrounding to cellulose of *Miscanthus* stalks would be decreased in the pretreatment process. The perfect crystal in the defect crystalline regions would be exposed. The crystalline regions would become distinct and the crystallinity index was increased after pretreatment. It also indicated that hydrolysis effect caused by guanidine and amino-guanidine catalysts was stronger in the amorphous regions than the crystalline regions. Thus, the crystallinity index of cellulose was correspondingly increased. The crystallinity index of cellulose

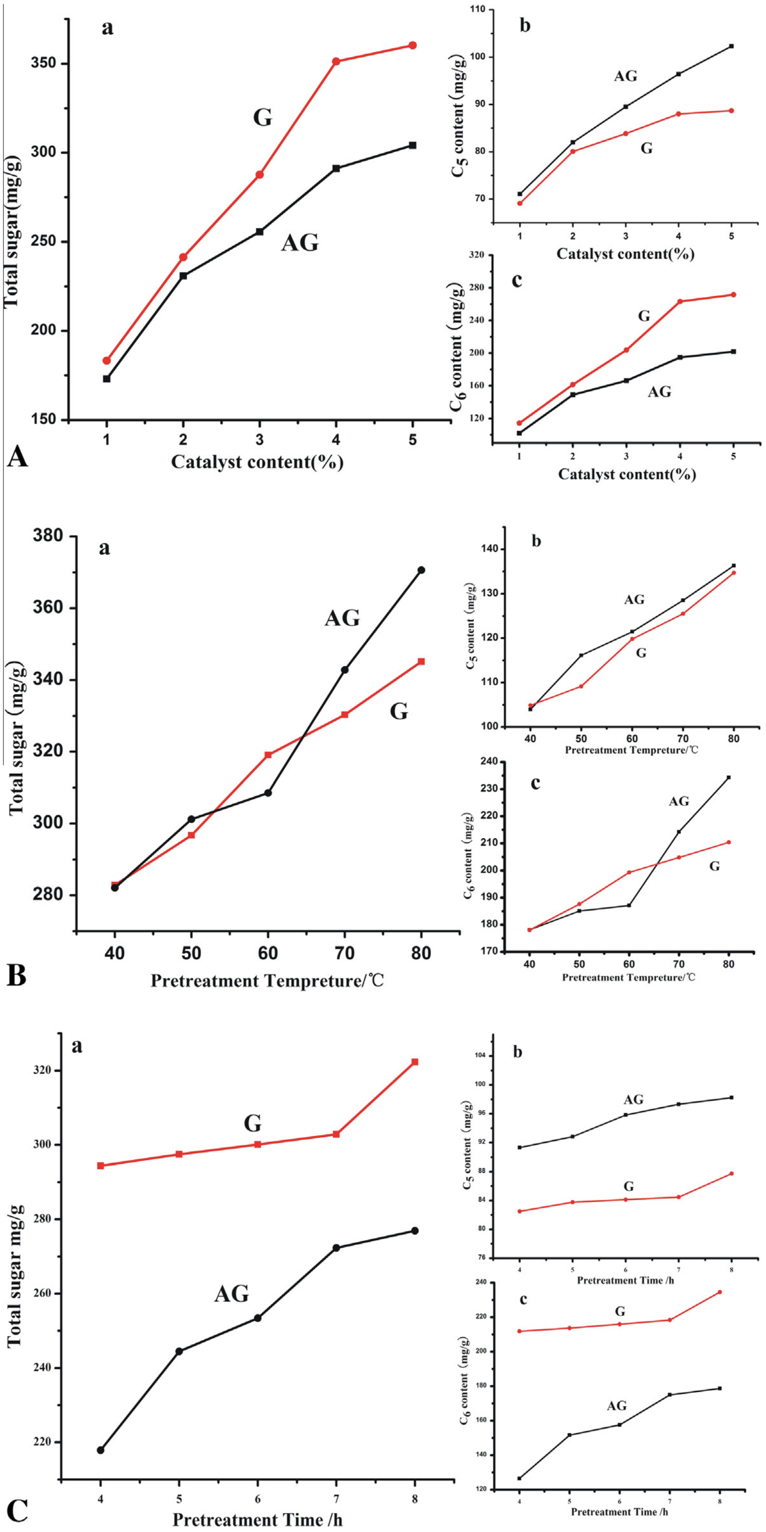

Fig. 1. Effects of pretreatment by guanidine (G) and amino-guanidine (AG) on sugar yield in enzymolysis process. (A)–(C) is the influence of catalyst loadings, pretreatment temperature and pretreatment time on sugar yield, respectively. a–c is the influence of pretreatment on the total sugar, pentose, hexose yield, respectively.

of *Miscanthus* stalks was significantly and mainly influenced by catalyst loadings, as shown in Supplemental Table S1.

3.3. FTIR analysis

FTIR performance of the pretreated *Miscanthus* stalks in Section 3.1 was carried out to study the influence of catalysts on the pretreatment results of *Miscanthus* stalks. The FTIR spectrum (Supplemental Fig. S1) shows that IR absorption was gradually shifted to large wave number region with increasing the catalyst loadings. It indicated that the strength of the hydrogen bonds between cellulose molecules was gradually attenuated with increasing catalyst loadings. The crystalline structure of cellulose of *Miscanthus* stalks was destroyed (Yoshida et al., 2008). The FTIR result showed that guanidine catalysts were favor of removing lignin and degrading hydrogen bonds, in agreement with the previous report, in which ammonia water was used to pretreat stalks (Laskar et al., 2013). It indicated that alkali with amino groups was suitable catalyst to remove lignin.

3.4. SEM analysis

SEM (Supplemental Fig. S2(a)) shows the surface of *Miscanthus* stalks without pretreatment was smooth, compacted and ordered, and after pretreatment, the surface of *Miscanthus* stalks was hollow, irregular, and corroded. The structure became loosen and unordered. It indicated that the original structure of *Miscanthus* stalks could be destroyed effectively by amino-guanidine and guanidine in pretreatment process. The surface area was significantly increased. The accessibility of enzyme was correspondingly increased. By comparing Fig. S2(b) with (c), based on the corrosion extent, the pretreatment result of the guanidine catalyst was better than that of the amino-guanidine catalyst, in agreement with the result in Section 3.1.

3.5. TG analysis

TG characterization of the pretreated *Miscanthus* stalks in Section 3.1 was performed to study the thermal property of *Miscanthus* stalks before and after pretreatment by catalysts. The TG curves of the samples pretreated by the amino-guanidine and the guanidine were shown in Fig. 2A and B, respectively. The TG curve of the sample without catalytic pretreatment was marked as r. The TG curves of the samples pretreated by 1–5% amino-guanidine catalysts and guanidine catalysts were marked as a–e and f–j, respectively, as shown in Fig. 2.

The weight loss in the range of 0–150 °C was due to the evaporation of water and some small molecules, as shown in Fig. 2. The trend of TG curves of *Miscanthus* stalks with and without catalytic pretreatment seemed to be the same in this range with weight loss of about 5%. In the temperature range of 150–340 °C, the weight for the sample without pretreatment was rapidly lost about 35%, while the weight for the samples with catalytic pretreatment began to be lost around 250 °C. It was because lignin was partly removed after pretreatment by alkali catalysts. The cellulose content in samples, as well as the relative crystallinity of cellulose, was increased. Thus, the thermal decomposition temperature was correspondingly increased. After 340 °C, the samples without pretreatment was weight lost slowly again. However, the percentage was smaller than that of samples with catalytic pretreatment. In this range, lignin is the dominated component for weight loss. The lignin content in the samples without pretreatment was relatively high. Thus, their thermal decomposition temperature was increased because of the highly cross-linked net-like structure of lignin (Vanderghem et al., 2011). After 360 °C, the samples with catalytic pretreatment was weight lost slowly again. The weight loss at the same temperature was increased with increasing catalyst loadings. It indicated that the lignin can be removed by increasing catalyst loadings (Chen and Kuo, 2011). The trend of TG curves of samples with pretreatment by the guanidine and the amino-guanidine was similar. However, the initial temperature of rapid weight loss of cellulose is different in different catalytic pretreatment. The initial temperatures of cellulose decomposition of *Miscanthus* stalks pretreated by the amino-guanidine and the guanidine were 250 °C and 260 °C, respectively. It indicated that more lignin was removed in the samples pretreated by the guanidine catalyst than by amino-guanidine catalyst. Thus, the relative crystallinity index and contents of cellulose were correspondingly higher for the guanidine catalyst. It would cause the higher initial decomposition temperature.

3.6. Pretreatment mechanism

A pretreatment mechanism of *Miscanthus* stalks by guanidines or amino-guanidines can be postulated based on the results of pretreatment and characterization. First, basic hydrolysis of the catalysts resulted in the removal of lignin and the break among cellulose, hemicelluloses, and lignin. The basicity of guanidines or amino-guanidines is created through their protonation and dissociation of water molecules to hydroxide ions: one guanidine reacts with three water giving 3 OH^- and amino-guanidine giving 4.

The guanidines exist in conjugated Bronsted acid form. The dissociated hydroxide group (OH^-) plays a role of hydrolysis. The stronger the base is, the weaker the conjugated Bronsted acid is, and in verse. The acidity of protonated amino-guanidines is stronger than that of protonated guanidines, leading to more hemicelluloses chains destruction of *Miscanthus* stalks pretreated by the amino-guanidine catalyst. Thus, the pentose contents obtained after enzymolysis of *Miscanthus* stalks pretreated by the amino-guanidine catalyst was higher than that by the guanidine catalyst. Second, the formation of new hydrogen bonds between guanidine catalyst and crystal cellulose is the main reason to break the old hydrogen bonds between cellulose chains. The net of hydrogen bonds of cellulose was substituted by the transitional bond formation, such as cellulose-O-H$\cdots$$N_3CH_5$ and cellulose-O-H$\cdots$$N_4CH_6$, through hydroxide groups of intramolecular or intermolecular hydrogen bonds between guanidine and cellulose long-chains. The cellulose long-chains would lose their bonding force to cause a disordered state. The old hydrogen bonds of cellulose were destroyed. The FTIR results illustrated that the IR absorption peaks was blue shifted. It was because the transitional hydrogen bonds were formed. The non-crystalline region of cellulose was more easily to be form transitional hydrogen bonds than the crystalline region.

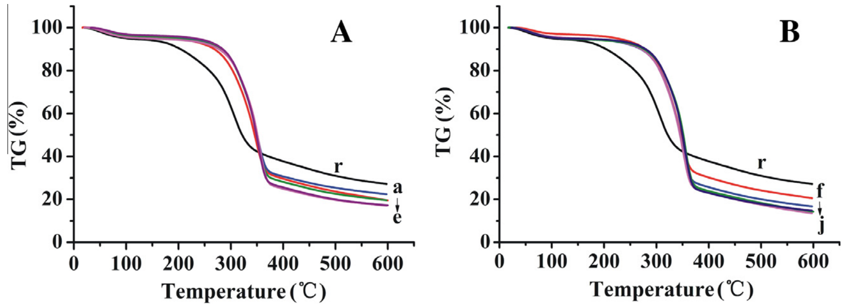

Fig. 2. TG curves of *Miscanthus* stalks pretreated by catalysts with different contents. (A) Amino-guanidine pretreatment, (B) guanidine pretreatment. Pretreatment condition: temperature 60 °C, catalyst loadings 3%, time 6 h.

4. Conclusions

The guanidine and amino-guanidine catalysts were used to pretreat *Miscanthus* stalks, and these catalysts not only could remove lignin but also could effectively destroy the interaction of hydrogen bonds between celluloses. The general pretreatment effect of the guanidine catalyst was better than that by the amino-guanidine catalyst. However, if the pretreatment temperature was over 65 °C, the pretreatment effect of the amino-guanidine catalyst was better. Because these catalysts are a class of environmentally friendly catalysts, they are considered as perspective catalysts in application of pretreatment of biomass stalks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.11.110.

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